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The goal of this investigation is to determine whether modulation of NF-κB activity by genetic engineering of DCs can affect DC longevity and T cell stimulatory functions. The specific aims are: 1) To determine the effect of expression of constitutively-active IKKβ (IκB kinase) on nuclear NF-κB levels in DCs, and on the longevity of DCs. 2) To determine the effect of IKKβ expression on in vitro and in vivo T cell priming by DCs. Results from these studies may thus lead the way for development of approaches for enhancing DC survival and function, with the goal of generating potent anti-tumor immunity.

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Introduction

The goal of this investigation is to determine whether modulation of NF-κB activity by genetic engineering of dendritic cells (DCs) can affect DC longevity and T cell stimulatory functions. The specific aims are: 1) To determine the effect of expression of constitutively-active IKKβ (IκB kinase) on nuclear NF-κB levels in DCs, and on the longevity of DCs. 2) To determine the effect of IKKβ expression on in vitro and in vivo T cell priming by DCs. Results from these studies may thus lead the way to development of approaches for enhancing DC survival and function, with the goal of generating potent anti-tumor immunity.

Body

As mentioned in the last report, we first generated an appropriate retroviral construct for performing the proposed studies. A constitutively-active mutant the NF-κB activating IKKβ kinase (CA-IKKβ) was subcloned into the MIG retroviral vector (to generate MIG-IKK). This vector also expresses the GFP protein using an IRES sequence to allow detection of infected cells. MIG-IKK was then transfected into the retroviral packaging line BOSC. Retroviral supernatants were collected 48hr and 72hr after transfection.

Also, as mentioned previously, MIG-IKK virus was sufficient to activate NF-κB in mouse fibroblasts. Using the MIG control virus, we set about optimizing conditions for retroviral infection of mouse bone marrow (BM)-derived DCs. BM precursors were cultured in the presence of GM-CSF containing supernatant for 6 days. An infection rate of 40-70% GFP-positive DCs was obtainable when using the control MIG virus. In distinct contrast, infection with MIG-IKK resulted in no more than 1-2% infection, while the same virus readily infected mouse fibroblasts. These results indicate that infection with MIG-IKK likely leads to reduced survival and proliferation of DCs. Thus, NF-κB activation by CA-IKK is apparently detrimental to DCs (but not fibroblasts). These results are significant because they indicate that optimal levels of NF-κB are essential for DC viability and proliferation i.e., either hypo- or hyper-activation of NF-κB in DCs can be detrimental. However, our previous studies have shown that NF-κB is essential for survival of mature DCs. The key question is therefore how to reconcile our previous results with our new findings?

We believe that NF-κB activation likely inhibits proliferation and survival of *developing* DCs but not *mature* DCs. It is noteworthy that only dividing cells can be subjected to retroviral infections. Thus, retroviral infections are performed on proliferating BM precursors during their differentiation into DCs. Consequently, CA-IKK mediated NF-κB activation occurs in developing DC, which is likely detrimental for them. Based on these new findings, we want to specifically activate NF-κB in non-dividing mature DCs by CA-IKK expression. To this end, we will simultaneously attempt 3 different approaches, which are described next. Importantly, while the technical approach is being modified, the specific aims of this award remain unchanged.

1) Infection of BM cells with retroviruses conferring inducible expression of CA-IKK. The best-characterized system of this type allows expression using a retrovirus with a doxycycline-inducible promoter (this system is available commercially). Using this approach, DCs will be infected as described above. When non-dividing mature DCs are obtained at the end of the 6-day culture period, doxycycline will be added to induce CA-IKK expression. In this manner, detrimental effect of CA-IKK expression on developing DCs can be avoided. The testing of these DCs, however, will be performed as previously proposed.

2) Adenovirus-mediated expression of CA-IKK. Adenoviruses do not require cell proliferation for infection. Therefore, this approach can also be used to express CA-IKK

specifically in non-dividing mature DCs. Adenovirus-expression systems are also available commercially.

3) Nucleofection of mature DCs. A transfection procedure called Nucleofection has recently been developed Amaxa Inc. This procedure allows transfection of cells that are resistant to commonly used transfection methods, including primary and non-dividing cells. Contrary to classical electroporation, the DNA is directly transported into the nucleus. We have already tried many commonly used methods for transfecting DCs, but without success. We are therefore quite excited about testing this new method as a way to introduce CA-IKK into non-dividing mature DCs.

Key research accomplishment:

Our studies indicate that optimal levels of NF-κB are essential for DC development i.e., either hypo- or hyper-activation of NF-κB in DCs can be detrimental. Thus, NF-κB levels must be under very tight control during DC development.

Reportable Outcomes

1) Degrees obtained: Dr. Ye Zheng received his Ph.D. during the last funding period. His studies were supported in part by this award.

2) Funding applied: An R01 application was submitted to the National Institutes of Health based in part on studies supported by this award.

Conclusions

Our studies demonstrate that developing DCs are exquisitely sensitive to levels of NF-κB. These finding have led to a change in the experimental approach (as discussed above), which would allow better determination of how NF-κB levels in mature DCs impact T cell priming. During the next funding cycle, we will determine how expression of CA-IKKβ in mature DCs impacts their survival and T cell responses in vitro and in vivo. Of note, these studies will not only help determine the feasibility of our approach i.e., potentiating T cell responses against tumor cells, but may also develop novel technologies to modulate gene expression in DCs. Application of these new approaches may have wide-ranging applications in the areas of immune modulation of cancer.